IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
REQUEST FOR FILING APPLICATION UNDER RULE 53(b)

Pursuant to 37 CFR 1.53(b), please file a ☐ continuation/☒ divisional
of the pending prior PATENT APPLICATION
Inventor: CAVAZZA
Serial No. 09/446,806
Filed: December 28, 1999

JC685 U.S. PTO 07/28/00

Atty Dkt.: 2801-21
C# M#
Date: July 28, 2000
Group: 1617
Examiner: Kim

JC586 U.S. PTO 09/628345 07/28/00

For: **PHARMACEUTICAL COMPOSITIONS COMPRISING ALKANOYL L-CARNITINE IN COMBINATION WITH A STATINE FOR TREATING PATHOLOGIES BROUGHT AOUT BY AN ALTERED LIPID METABOLISM**

Assistant Commissioner for Patents
Washington, DC 20231
Sir:

This request for filing under Rule 53(b) is made by the following named inventor(s) (using the above-identified title):
Inventor(s): CAVAZZA

☒ Attached is a true copy of the prior application as originally filed including the specification, claims, Oath/Declaration and drawings (if any) and abstract (if any). No amendments (if any) referenced in the Oath or Declaration filed to complete the prior application introduced new matter.

☒ Priority is hereby claimed under 35 USC 119 based on the following foreign applications, the entire content of which is hereby incorporated by reference in this application:

Application Number	Country	Day/Month/Year/Filed
PCT/IT98/00163	PCT	18 June 1998
RM97A000390	Italy	1 July 1997

☐ certified copy(ies) of foreign application(s) attached or
☐ already filed on _____ in prior appln. no. _____ filed _____
☒ already filed in PCT/IT98/00163 filed June 18, 1998

☒ The prior application is assigned to Sigma-Tau Industrie Farmaceutiche Riunite S.p.A..

☒ Power of Attorney has been granted to Arthur R. Crawford et al, Reg. No. 25,327 of Nixon & Vanderhye P.C., 1100 N. Glebe Rd., 8th Flr, Arlington, VA 22201.

☒ Address all future communications to: Nixon & Vanderhye P.C., 1100 N. Glebe Rd., 8th Floor, Arlington, VA 22201

☒ Please amend the specification by inserting before the first line --This is a division of application Serial No. 09/446,806, filed December 28,1999, now U.S. patent No. , which is a 35 U.S.C. §371 of PCT/ IT98/00163 filed June 18, 1998 the entire content of which is hereby incorporated by reference in this application.--

☒ The Examiner's attention is directed to the prior art cited in the parent application by applicant and/or Examiner for the reasons stated therein.

☒ Please enter the attached and/or below preliminary amendment **prior** to calculation of filing fee:
Attached Information Disclosure Statement

☒ The entire disclosure of the prior application above-referenced is considered as being part of the disclosure of this new application and is hereby incorporated by reference therein.

The PTO did not receive the following listed item(s) Amendment Rec'd 11/

FILING FEE IS BASED ON CLAIMS AS FILED LESS ANY HEREWITH CANCELED

Basic Filing Fee					$	690.00
Total effective claims	13	- 20 (at least 20) =	0	x $ 18.00	$	0.00
Independent claims	5	- 3 (at least 3) =	2	x $ 78.00	$	156.00
If any proper multiple dependent claims now added for first time, add $260.00 (ignore improper)					$	0.00
				SUBTOTAL	$	846.00
If "small entity," then enter half (1/2) of subtotal and subtract					-$(	0.00)
				SECOND SUBTOTAL	$	846.00
Assignment Recording Fee ($40.00)					$	0.00
				TOTAL FEE ENCLOSED	$	846.00

Any future submission requiring an extension of time is hereby stated to include a petition for such time extension. The Commissioner is hereby authorized to charge any deficiency in the fee(s) filed, or asserted to be filed, or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our **Account No. 14-1140.** A duplicate copy of this sheet is attached.

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NIXON & VANDERHYE P.C.
By Atty: Arthur R. Crawford, Reg. No. 25,327

Signature: _____

The present invention relates to a pharmaceutical composition for the prevention and treatment of cardiovascular diseases caused by abnormal lipid metabolism.

Cardiovascular diseases related to abnormal lipid metabolism are very frequent in industrialised countries. In Italy, for instance, they account for more than 40% of the overall mortality (Capocaccia R., Farchi G., Prati S. *et al.*: La mortalità in Italia nell'anno 1989. Rapporto ISTISAN 1992/22). Our knowledge of the relationships between cholesterol and coronary heart disease stem from epidemiological studies conducted over the past few years. The conclusions reached in these studies indicate that the development of severe coronary atherosclerosis and coronary heart disease are closely correlated with serum cholesterol levels (McGill H.C. Jr. *et al.*: The International Atherosclerosis Project. Lab. Invest. 18: 463-653, 1968; Keys A.: Seven Countries: Death and Coronary Heart Disease. Harvard University Press, Cambridge, 1980).

Correction of eating habits through suitable diet is invariably the first measure adopted in cases of hyperlipidaemia. Satisfactory results are not always achieved, however, owing to widespread

intolerance of strict dietary discipline, to the severity of the hypercholesterolaemia, or to genetic-type resistance.

To achieve the desired results in these patients, i.e. normalisation of blood levels of triglycerides and cholesterol, pharmacological treatment has to be resorted to. Hypolipaemic drugs fall into two categories: those which above all reduce cholesterol and those which mainly reduce triglycerides.

The former group of drugs includes the statins, probucol and resins, while the latter group includes the fibrates, nicotinic acid and fatty acids belonging to the omega-3 series.

The statins (lovastatin, simvastatin, provastatin, fluvastatin, and the like) are inhibitors of hydroxy-methyl-glutaryl-coenzyme A (HMG-CoA) reductase. By inhibiting this enzyme, they reduce the hepatic synthesis of cholesterol (Lancet 1994; 334: 1383-1389). To compensate for the reduction of intracellular cholesterol the liver cell produces several receptors for LDL and VLDL lipoproteins, which are thus removed from the bloodstream.

The statins also give rise to reduced intestinal absorption of cholesterol of dietary origin and to a reduced output of apoprotein B present in low-density lipoproteins (LDL).

The statins are drugs which are better tolerated than the other anticholesterolaemic agents, but are not without drawbacks,

the side effects most commonly induced by these drugs being gastrointestinal disorders, skin rashes and headache.

A number of patients have also reported sleep disorders (EJ Schaffer, N Engl J Med, 319; 1222, 1988; Lancet, 339: 547, 29 February 1992), while, in 1-2% of patients taking high doses of statins, an at least 3-fold increase in plasma aminotransferase activity has been noted compared to baseline values, which may even require discontinuation of the treatment.

In addition, it has been reported that though the statins lead to a reduction in the number of deaths due to coronary heart disease, an increase has been observed, in treated patients, of deaths caused by other events such as tumours or trauma (Davey-Smith G., Song F., Sheldon T.A.: Cholesterol lowering and mortality: the importance of considering initial level at risk. BMJ, 1993; 306: 1367-1373; Ravnshov U.: Cholesterol lowering trials in coronary heart disease: frequency of citation and outcome. BMJ 1992; 305: 15-19). The results of experiments in animals and human subjects have suggested that, to reduce cholesterol levels, pharmacological treatment with statins should be given only to patients at high risk for coronary disease in the short term (JAMA, 1996; 275: 55-60).

Equally well known is the antitriglyceridaemic and anticholesterolaemic effect of a number of alkanoyl carnitines,

particularly acetyl L-carnitine. U.S. Patent 4,268.524 describes a therapeutic method for increasing high-density lipoprotein (HDL) levels so as to selectively reduce the HDL:(LDL + VLDL) ratio in the plasma of patients at risk for cardiovascular disease, in which this ratio is abnormally high; the method comprises administering 5-50 mg/kg/day of alkanoyl carnitine or one of its pharmacologically acceptable salts.

It has now been found, unexpectedly, that the co-ordinated use - this term being defined precisely here below - of an alkanoyl L-carnitine in which the linear or branched alkanoyl has 2-6 carbon atoms, or of one of its pharmacologically acceptable salts, and a statin enables an enhanced effect on the anticholesterolaemic and antitriglyceridaemic action to be achieved as compared to the separate, independent administration of the two active ingredients. This enables the same therapeutic results to be achieved using lower doses of statins, thus making for a marked reduction in their toxic and side effects.

The well-known lack of toxic and side effects of the alkanoyl L-carnitines and the use of lower doses of statins as compared to the routine doses (10-40 mg/day) makes the co-ordinated use as per the invention particularly useful and safe both for the treatment of hypercholesterolaemic and/or hypertriglyceridaemic

patients at high risk for cardiovascular disease in the short, medium or long term and for the prevention of such diseases.

As a result of the above-mentioned synergistic effect, it has been found, in fact, that the statin dose can be reduced to 5-20 mg/day, whereas the alkanoyl L-carnitine dose can be reduced to 2-30 mg/kg/day.

According to the present invention, what is meant by "co-ordinated use" of the afore-mentioned compounds is *either* their co-administration, i.e. the substantially simultaneous administration of one of the aforesaid alkanoyl L-carnitines, or one of their pharmacologically acceptable salts, and of a statin, *or*, indifferently, the administration of a composition comprising a combination or mixture of the aforesaid active ingredients, optionally in addition to suitable excipients.

The scope of the present invention encompasses therefore both the co-administration of one of the aforesaid alkanoyl L-carnitines, or one of their pharmacologically acceptable salts, together with a statin, and orally or parenterally administrable pharmaceutical compositions. comprising a mixture of the two active ingredients.

Preferably the statin is selected from the group comprising lovastatin, simvastatin, provastatin and fluvastatin, while the alkanoyl L-carnitine is selected from the group comprising acetyl,

propionyl, butyryl, valeryl and isovaleryl L-carnitine or one of their pharmacologically acceptable salts.

Even more preferably, the statin is simvastatin and the alkanoyl L-carnitine propionyl L-carnitine or one of its pharmacologically acceptable salts.

What is meant by pharmacologically acceptable salt of an alkanoyl L-carnitine is any salt of the latter with an acid that does not give rise to unwanted toxic or side effects.

These acids are well known to pharmacologists and to experts in pharmacy.

Non-limiting examples of pharmacologically acceptable salts of alkanoyl L-carnitines are chloride, bromide, orotate, acid aspartate, acid citrate, acid phosphate, fumarate and acid fumarate, lactate, maleate and acid maleate, acid oxalate, acid sulphate, glucose phosphate, tartrate and acid tartrate.

One preferred composition, in unit dosage form, is a composition comprising statin 5-10 mg and alkanoyl L-carnitine 100-1000 mg.

The enhanced effect of alkanoyl L-carnitine and statin has been confirmed, for example. by the results of a clinical study which are given here below.

CLINICAL STUDY

Eight hypertriglyceridaemic patients (3 males and 5 females) were recruited for the study, with a mean age of 65 years (range: 52-70), presenting mean triglyceridaemia values of 213.0 ± 21.18 mg% and mean cholesterolaemia values of 158.1 ± 25.90 mg%, who were put on a dietary regimen consisting in 40 Kcal/kg, 1.2 g/kg proteins and 1.4 g/kg lipids daily.

After baseline determinations of cholesterolaemia, triglyceridaemia, bilirubinaemia, alkaline phosphatase, protidaemia, GOT and GPT, all subjects took propionyl L-carnitine 2 g/day os for 60 days, which was discontinued on day 61 for a 30-day wash-out period. Simvastatin was then administered at a dose of 10 mg/day os in the evening for 30 days. followed by a further 30-day wash-out period and then by another 30-day period during which propionyl L-carnitine 1.5 g/day os and simvastatin 5 mg/day os were administered simultaneously.

Blood-chemistry tests were performed before and after each course of drug treatment and at the end of each wash-out period. The data obtained were subjected to statistical analysis using Student's t-test for paired data (Table 1).

TABLE 1. - Experimental protocol/baseline study

Treatment with propionyl L-carnitine 2 g/day os	Wash-out	Treatment with simvastatin 10 mg/day os	Wash-out	Treatment with propionyl L-carnitine 1.5 g/day os + simvastatin 5 mg/day os
60 days	30 days	30 days	30 days	30 days
Blood-chemistry tests	Blood-chemistry tests	Blood-chemistry tests	Blood-chemistry tests	Blood-chemistry tests

TABLE 2. - Statistical analysis of results

Cholesterolaemia

Time	Propionyl L-carnitine	Simvastatin	Propionyl L-carnitine + simvastatin
0	-	-	-
30 days	$p < 0.4795$	$p < 0.0585$	$p < 0.0389$
60 days	$p < 0.0198$	-	-

Triglyceridaemia

0	-	-	-
30 days	$p < 0.3671$	$p < 0.0247$	$p < 0.0015$
60 days	$p < 0.0272$	-	-

RESULTS

Owing to the low dose of simvastatin and the substantial non-toxicity of propionyl L-carnitine, no side effects attributable to the drugs used were detected during the study period. All patients completed the study according to the procedures described. As regards triglyceridaemia during the period of treatment with propionyl L-carnitine, only a slight, statistically non-significant reduction ($p < 0.3671$) was recorded after 30 days as compared to baseline values and this reduction proved statistically significant ($p < 0.0272$) only after 60 days' treatment with propionyl L-carnitine. During the wash-out period the mean triglyceridaemia value recorded was 202.5 ± 9.71 mg%, whereas the mean baseline value was 213.0 ± 21.11 mg% (Table 2).

After treatment with simvastatin a statistically significant reduction in triglycerides was recorded as compared to basal values ($p < 0.024$), with a mean post-treatment triglyceride value of 193.8 ± 22.63 mg% (Table 2).

At the end of the wash-out period following simvastatin treatment, the mean triglyceride value was 205.37 ± 13.98 mg%. At the end of the treatment with propionyl L-carnitine and simvastatin administered simultaneously, the mean triglyceride value was 146.62 ± 27.93 mg% and presented a statistically significant reduction as compared to baseline conditions ($p <$

0,0001). On comparing statistically the triglyceridaemia values recorded after treatment with propionyl L-carnitine and simvastatin, respectively, administered alone, with those recorded after treatment with the combination, the following significance values were found: $p < 0.167$ (propionyl L-carnitine vs simvastatin); $p < 0.00031$ (propionyl L-carnitine vs propionyl L-carnitine + simvastatin); $p < 0.0004$ (simvastatin vs propionyl L-carnitine + simvastatin) (Table 2).

As regards cholesterolaemia values, which were within normal limits in baseline conditions (mean 158.12 ± 25.90 mg%), statistically significant reductions were recorded in the comparison among values recorded at the end of the first wash-out period (158.37 ± 25.90 mg%), after treatment with simvastatin (156.75 ± 22.82) and in the comparison between baseline values (158.12 ± 25.90 mg%) and those obtained after the period of treatment with the combination of propionyl L-carnitine and simvastatin (135.51 ± 15.2 mg%) ($p < 0.0038$) (Table 2).

The results of this clinical study provide significant evidence in support of the enhanced effect of alkanoyl L-carnitine and statin, which constitutes the basis of the present invention. The data obtained, in fact, demonstrate without doubt that the pharmacological combination of propionyl L-carnitine and simvastatin presents a superior cholesterolaemia-lowering and

triglyceridaemia-lowering effect as compared to the administration of propionyl L-carnitine and simvastatin separately and independently. This allows a drastic reduction in the daily dose of simvastatin (from 10 mg/day to 5 mg/day), which thus falls below the threshold at which the above-mentioned unwanted toxic and side effects usually manifest themselves.

CLAIMS

1. An orally or parenterally administrable pharmaceutical composition which comprises an alkanoyl L-carnitine wherein the linear or branched alkanoyl group has 2-6 carbon atoms, or one of its pharmacologically acceptable salts, and a statin.

2. The composition of claim 1, wherein the alkanoyl L-carnitine is selected from the group comprising acetyl L-carnitine, propionyl L-carnitine, butyryl L-carnitine, valeryl L-carnitine and isovaleryl L-carnitine.

3. The composition of claim 1, wherein the statin is selected from the group comprising lovastatin, simvastatin, provastatin and fluvastatin.

4. The composition of claim 1, wherein the pharmacologically acceptable salt of the alkanoyl L-carnitine is selected from the group comprising chloride, bromide, orotate, acid aspartate, acid citrate, acid phosphate, fumarate and acid fumarate, lactate, maleate and acid maleate, acid oxalate, acid sulphate, glucose phosphate, tartrate and acid tartrate.

5. The composition of claim 1, wherein the statin is simvastatin and the alkanoyl L-carnitine is propionyl L-carnitine or one of its pharmacologically acceptable salts.

6. The composition of any of the preceding claims with a cholesterolaemia-lowering and triglyceridaemia-lowering action for the treatment of diseases caused by abnormal lipid metabolism.

7. The composition of claim 6 for the treatment of cardiovascular, thrombotic, atherosclerotic diseases and of peripheral vascular diseases.

8. The composition of claim 1, in unit dosage form, comprising of 5-10 mg of statin and 100-1000 mg of alkanoyl L-carnitine.

9. Co-ordinated use of an alkanoyl L-carnitine wherein the linear or branched alkanoyl group has 2-6 carbon atoms, or of one of its pharmacologically acceptable salts, and a statin, for the prevention and treatment of diseases caused by abnormal lipid metabolism.

10. Use of an alkanoyl L-carnitine wherein the linear or branched alkanoyl group has 2-6 carbon atoms, or of one of its pharmacologically acceptable salts, and a statin, to produce a medicament for the prevention and treatment of diseases caused by abnormal lipid metabolism.

11. The use of claims 9 or 10, in which the statin is selected from the group comprising lovastatin, simvastatin, provastatin and fluvastatin and the alkanoyl L-carnitine is selected from among the group comprising acetyl L-carnitine, propionyl L-carnitine, butyryl L-carnitine, valeryl L-carnitine and isovaleryl L-carnitine or one of their pharmacologically acceptable salts.

Case No. _____ Nixon & Vanderhye P.C. (12/97)

RULE 63 (37 C.F.R. 1.63)
DECLARATION AND POWER OF ATTORNEY
FOR PATENT APPLICATION
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

As a below named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name, and I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled: ***"Pharmaceutical compositions for treating pathologies brought about by an altered lipid metabolism"***

the specification of which (**check** applicable **box(s)**):

is attached hereto

was filed on _____ as U.S. Application Serial No. _____

X was filed as PCT International application No. **PCT/IT 98/00163** on **June 18, 1998**

and (if applicable to U.S. or PCT application) was amended on _____

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose information which is material to the patentability of this application in accordance with 37 C.F.R. 1.56. I hereby claim foreign priority benefits under 35 U.S.C. 119/365 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed or, if no priority is claimed, before the filing date of this application:

Priority Foreign Application(s):

Application Number	**Country**	**Day/Month/Year Filed**
RM97A000390	ITALY	**01.07.1997**

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below.

Application Number	**Date/Month/Year Filed**

I hereby claim the benefit under 35 U.S.C. 120/365 of all prior United States and PCT international applications listed above or below and, insofar as the subject matter of each of the claims of this application is not disclosed in such prior applications in the manner provided by the first paragraph of 35 U.S.C. 112, I acknowledge the duty to disclose material information as defined in 37 C.F.R. 1.56 which occurred between the filing date of the prior applications and the national or PCT international filing date of this application:

Prior U.S./PCT Application(s): Application Serial No.	**Day/Month/Year Filed**	**Status: patented pending, abandoned**

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon. And I hereby appoint **NIXON & VANDERHYE P.C., 1100 North Glebe Rd., 8th Floor, Arlington, VA 22201-4714, telephone number (703) 816-4000 (to whom all communications are to be directed)**, and the following attorneys thereof (of the same address) individually and collectively my attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith and with the resulting patent: Arthur R. Crawford, 25327; Larry S. Nixon, 25640; Robert A. Vanderhye, 27076; James T. Hosmer, 30184; Robert W. Faris, 31352; Richard G. Besha, 22770; Mark E. Nusbaum, 32348; Michael J. Keenan, 32106; Bryan H. Davidson, 30251; Stanley C. Spooner, 27393; Leonard C. Mitchard, 29009; Duane M. Byers, 33363; Jeffry H. Nelson, 30481; John R. Lastova, 33149; H. Warren Burnam, Jr. 29366; Thomas E. Byrne, 32205; Mary J. Wilson, 32955; J. Scott Davidson, 33489; Alan M. Kagen, 36178; William J. Griffin, 31260; Robert A. Molan, 29834; B. J. Sadoff, 36663; James D. Berquist, 34776; Updeep S. Gill, 37334.

Inventor's Signature: _____ Date: December 14, 1999

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FOR ADDITIONAL INVENTORS, check box ☐ and attach sheet with same information and signature and date for each.

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